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Structural adaptations in compressed articular cartilage measured by diffusion tensor imaging

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Summary

Objective: To demonstrate the use of diffusion tensor magnetic resonance micro-imaging to observe adaptations of collagen fibres to mechanical compression in articular cartilage.

Methods: Spin-echo and diffusion tensor images (156 × 156 μm in-plane resolution, 2 mm slice thickness) of bovine cartilage were obtained at a magnetic field of 7.0 T in relaxed and compressed states. The parameters determined were: T_2, maximum and mean diffusivity, direction of the maximum diffusion eigenvector and fractional anisotropy of diffusion.

Results: A correlation was found between the compressive strain applied to the cartilage and the change in both magnitude and direction of the maximum diffusivity. Compression resulted in a decrease in both the maximum and mean eigenvalues, particularly in the surface and transitional zones, while the change in orientation of the eigenvectors corresponding to maximum diffusion was greatest in the transitional region. In this region, the average orientation of the principal eigenvectors with respect to the normal to the articular surface increased by up to 40°, indicating that the collagen fibre bundles were oriented more parallel to the surface when compressed.

Conclusions: Diffusion tensor imaging can be used to monitor the changes in the direction of the collagen fibres due to compression. It may form the basis of a new non-invasive approach to functional evaluation of cartilage, with potential applications in the diagnosis and treatment of osteoarthritis.
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Key words: Articular cartilage, MRI, Diffusion tensor imaging, Compression, Collagen, Osteoarthritis.

Introduction

The primary function of articular cartilage is to provide a low friction joint surface, which can both transmit and help to distribute loads while attenuating peaks of stress. Prior to the development of magnetic resonance imaging (MRI) techniques, it was difficult to study the morphological structure of articular cartilage and its response to loading, without significantly altering the tissue[1–3]. However, recent publications have shown that the anisotropic diffusion of water in cartilage, as visualised by MRI using diffusion tensor imaging (DTI), reflects the orientation of the collagen fibres[4,5].

The structure of (uncompressed) bovine cartilage was studied by Jeffery *et al.*[1] using scanning electron microscopy (S.E.M.) and the results correlated with 'split line' patterns obtained by pricking the surface of the cartilage and using the direction of the resulting split to infer the surface fibre orientation. They confirmed earlier work using polarised light microscopy (PLM) by Benninghoff[6] which suggested that the collagen fibres curve from being approximately perpendicular to the bone at the subchondral plate, to being approximately parallel to the surface at the articular surface. A significant problem in using S.E.M. to image cartilage is the difficulty in visualising the collagen fibres when they are bound so closely with the proteoglycans (PGs). Jeffery *et al.*[1] partially removed the PGs by digestion with hyaluronidase after fixation in order to better visualise the collagen fibres. MRI has the advantage that it can be used to image the tissue in its natural state, without the need for sectioning, fixing, dehydration or removal of the PGs.

The anisotropic structure of collagen fibres in articular cartilage gives rise to a characteristic lamellar structure in magnetic resonance (MR) images, comprising light and dark bands that depend on the orientation of the normal to the articular surface with respect to the static magnetic field[7]. The banding arises predominantly from orientation dependence of the spin–spin relaxation time T_2[7,8], although the precise relationship between T_2 and collagen fibre structure and orientation is complex[7,9,10].

A number of authors have studied how the loading of cartilage affects the collagen structure. Kaab *et al.*[11] used cryopreservation of intact rabbit knee joints with subsequent fixation and S.E.M. of the tibial plateau, to demonstrate an increased thickness of the layer of collagen fibres that are almost parallel to the articular surface under load[3,12]. The thickness of this tangential surface layer depended on the force applied. They also observed bending and crimping of the collagen fibres in the transitional and upper radial

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zones of the cartilage. The observed changes in the collagen fibre structures depended on whether the cartilage was statically or cyclically loaded[3]. Glaser and Putz[2] showed that in bovine humeral head cartilage under static load, the collagen fibres tend to bulge away from the centre of the load. Fibres directly under the load formed a 'c' shape, which was in contrast to the sharper bending and crimping seen in the studies of rabbit cartilage by Kaab *et al.*[12].

There have been several published studies which have employed MRI techniques to investigate compressed cartilage. Rubenstein *et al.*[13] used spin-echo images to study the change in signal intensity across the cartilage induced by water loss and collagen fibre reorientation (especially in the radial zone) during compression. Regatte *et al.*[14] measured sodium and proton MR properties of bovine patella cartilage and demonstrated that ^{23}Na relaxation parameters are sensitive to PG depletion in uncompressed samples, while the proton spin–spin relaxation time T_2 becomes sensitive to PG depletion only after the sample is compressed. Shinar *et al.*[15] used ^{2}H double quantum filtered MRI of bovine cartilage (in which the water was replaced by D_2O) to demonstrate that water molecules bound to the collagen reflect the local fibre orientation. Their results were consistent with an increase in the thickness of the superficial region (in which collagen fibre orientation is primarily parallel to the articular surface) and provided some evidence for crimping of collagen fibres in the radial zone under load. Alhadlaq and Xia[16] measured profiles of the spin–spin relaxation time T_2 through canine cartilage for two orientations of the normal to the articular surface (0° and 55°) with respect to the static magnetic field. For both orientations, T_2 shortened on compression (predominantly due to water loss on consolidation) and for the 'magic angle' orientation of 55°, a laminar structure became apparent that was not evident in the uncompressed samples. Results were interpreted as evidence for an increase in both relative and absolute thickness of the superficial zone with a concomitant decrease in the radial zone. Nieminen *et al.*[17] investigated the relationship between MRI parameters and biomechanical properties of bovine cartilage. They reported correlations between Gd-DTPA (gadolinium-diethylenetriaminepentaacetic acid) enhanced T_1 and T_2 relaxation times and elastic moduli, claiming that 'frequently MRI parameters were able to explain from about 50% to 87% of the variation in biomechanical parameters'.

Diffusion of water in anisotropic tissues can be characterised by means of a 3×3 diffusion tensor, the principal eigenvalues and eigenvectors of which characterise the magnitude and direction of diffusion, respectively, in three-dimensional space[18]. The present study builds on previous work by Meder *et al.*[4] and Filidoro *et al.*[5], which suggests that the principal eigenvectors of the water diffusion tensor follow the collagen fibre directions. This is because, on the timescale of the DTI measurements, water molecules diffuse over distances of a few microns[4], which is much larger than the diameter ($\sim$40 nm) of the collagen fibrils themselves[19]. Consequently the diffusion of water in cartilage is restricted by the presence of the collagen fibre bundles. This restriction is greatest in a direction normal to the fibres, leading to a lower diffusivity in this direction and a correspondingly higher diffusivity parallel to the direction of the local fibre orientation. The aim of the research presented in this paper was to demonstrate the ability of DTI to visualise changes in water mobility and collagen fibre orientations due to compression and to compare the results with data obtained by less direct or more invasive techniques.

Materials and methods

SAMPLE PREPARATION

Bovine knee joints, from animals aged 18–30 months, were obtained fresh from a local abattoir on the morning of testing. Using a scalpel and saw, articular cartilage was removed from the medial condyle of the patellar (trochlear) groove on the femur, full depth, to include a portion of the underlying subchondral bone. Each rectangular sample (*ca.* 4×10 mm) was shaped to enable the sample orientation to be replicated and ensure imaging took place in approximately the same plane before and after compression. A total of ten individual specimens (each from a different knee joint) were employed in this study.

MRI

MRI experiments were undertaken on a 7.0 T vertical bore Bruker Avance nuclear magnetic resonance (NMR) spectrometer (Bruker BioSpin, Rheinstetten, Germany), equipped with a 1.1 T m^{-1} (110 G cm^{-1}) gradient set and a 15 mm 'birdcage' RF resonator. Samples were imaged in a 15 mm test tube surrounded by phosphate buffered saline. Teflon® plugs were employed to orient samples with

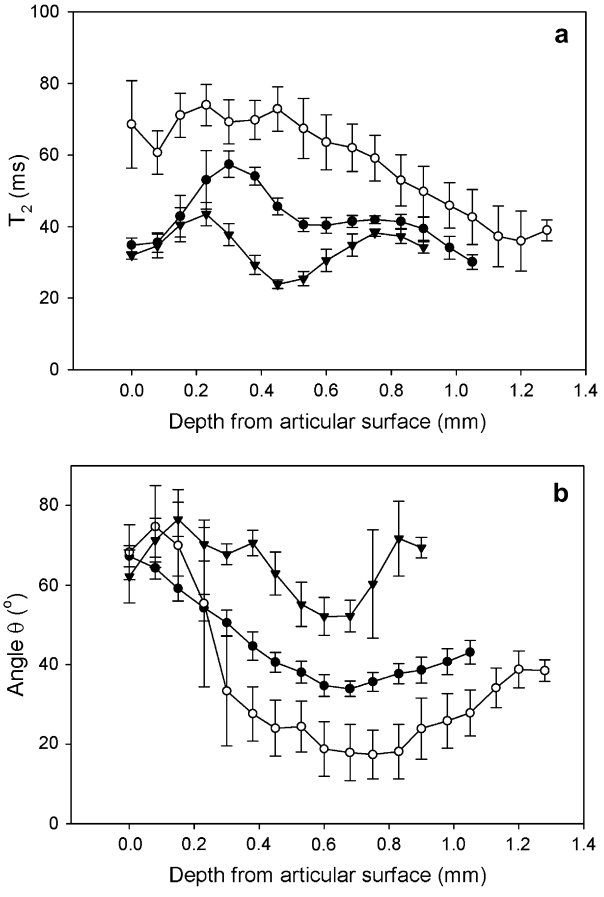

Fig. 1. Profiles of (a) T_2 and (b) diffusion eigenvector orientation θ for a typical sample before compression (○) and after compression by 18% (●) and 29% (▼) of the original cartilage thickness. The profiles are plotted against depth, measured from the articular surface of the cartilage and were obtained with the normal to the articular surface oriented at 55° ± 5° to the static magnetic field.

the normal to the articular surface at the 'magic angle' (55°) to the direction of B_0, in order to maximise the spin–spin relaxation time T_2 and ensure maximum signal from the sample at all depths. Sample orientation was checked from the images and adjusted if necessary.

Diffusion weighted images were acquired at 25.0 ± 0.1°C using a diffusion weighted pulsed gradient spin-echo sequence, with a recycle time (TR) = 2 s, an echo time (TE) of 12.6 ms, eight averages, a 2 mm slice thickness, and an in-plane resolution of 156 μm. Values of the diffusion gradient duration δ, diffusion gradient separation time Δ, and diffusion gradient strength G, were 2 ms, 8 ms and 0.7 T m^{-1}, respectively. A total of seven measurements were acquired for each diffusion tensor, six with the diffusion gradients applied in isotropically distributed directions, and one with no diffusion gradient. Spin-echo images (for computing T_2 profiles) were acquired using a TR of 2.5 s, a TE of 4.3 ms, 128 echoes and four averages. The same slice selection and in-plane resolution were used as for the diffusion images.

COMPRESSION

The cartilage samples were compressed in phosphate buffered saline, by placing them between two PVC (poly-vinyl chloride) plates, which could be clamped together with Nylon screws. Tightening the screws applied an arbitrary strain, which was measured from the MR images by comparing the uncompressed cartilage thickness with that of the compressed cartilage. The samples were compressed by up to 30% in one or two increments, each compression being followed by an equilibration time of at least an hour before further imaging. Previous measurements had confirmed that this was sufficient for samples to reach equilibrium as evidenced by no further detectable changes in T_2 values or the measured DTI parameters.

COMPUTATION AND REPRESENTATION OF DATA

Diffusion tensor images were calculated using in-house Matlab© code (The Mathworks, Natick, MA) written for the purpose. Thresholds were used to exclude signal from the surrounding saline, compression device and bone. Maximum eigenvalue, mean eigenvalue (defined as 1/3 of the trace of the diffusion tensor), eigenvector corresponding to the maximum eigenvalue, fractional anisotropy (defined as in Meder *et al.*[4]), and T_2 maps were calculated for the cartilage. The orientation of the eigenvector corresponding to the maximum eigenvalue (hereafter referred to as the principal eigenvector) was then expressed in terms of the angle θ between this eigenvector and the normal to the articular surface[4]. A section of the cartilage of approximately 50 pixels width and full depth was used to create an average profile of each of these parameters, through the depth of the cartilage. Other computational details have been described earlier[4].

Results

Profiles of average T_2 values and average principal eigenvector orientation θ through a typical bovine cartilage sample (Fig. 1) are plotted against depth, measured from the articular surface of the cartilage. The sample was imaged first in an uncompressed state, then when compressed by 18% of its original thickness and again when compressed by 29%. The T_2 profiles [Fig. 1(a)] show an overall decrease in the magnitude of T_2 across the sample, and an oscillation of the T_2 value under compression that becomes more pronounced with greater compression. Except in the superficial zone near the articular surface, the principal eigenvector profiles [Fig. 1(b)] show an increase in the average angle θ (between the direction of maximum diffusivity and the normal to the articular surface) with increasing compression. This implies that under compression, the principal direction of diffusion when averaged over all depths in the cartilage, became more aligned with the articular surface of the cartilage and corresponds to a thickening of the superficial region, in which the collagen fibres are aligned predominantly parallel to the articular surface.

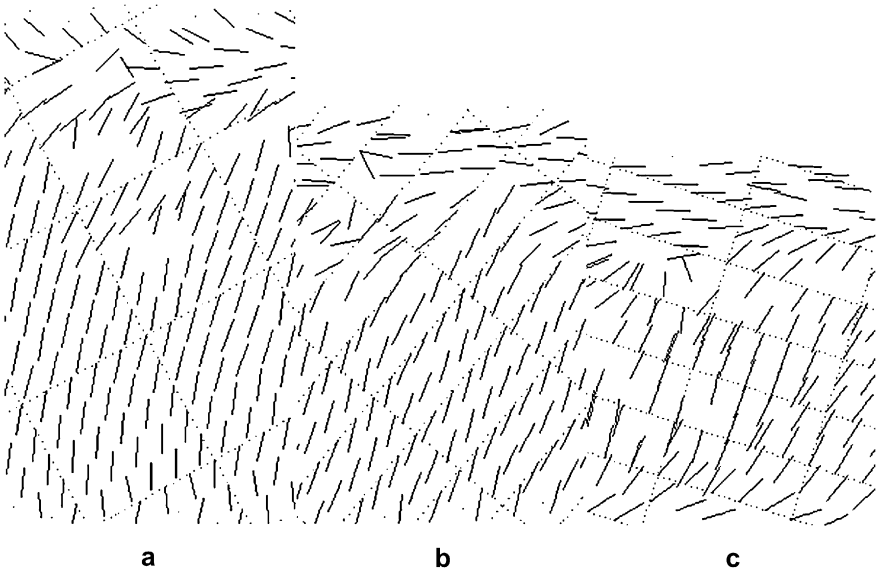

Fig. 2. Quiver plots of the same sample as Fig. 1, (a) before compression and after two subsequent compressions (b) by 18% and (c) by 29% of the original cartilage thickness, respectively. Each quiver is a projection onto the image plane of a unit vector in three dimensions representing the principal diffusion eigenvector at that point.

'Quiver plots' (Fig. 2) showing the direction of maximum water diffusivity for the same sample as Fig. 1, give an indication of the lateral spread of the principal eigenvector orientations θ over planes parallel to the articular surface as well as changes in average fibre orientation with depth and degree of compression. Each quiver is a projection onto the image plane of a unit vector in three dimensions representing the principal diffusion eigenvector at that point, which in turn reflects the local orientation with respect to the image plane of the collagen fibre bundles.

Average profiles of T_2, principal eigenvector orientation θ, maximum eigenvalue, mean eigenvalue and fractional anisotropy, both before and after compression, (Fig. 3) show the same trends as seen for the individual sample of Fig. 1. The profiles are plotted against normalised depth and were averaged across the seven samples used in this study that were compressed by 25% ± 5%, with error bars reflecting ±one standard deviation in the plotted parameter for the samples in both uncompressed and compressed states. The remaining three samples were not compressed sufficiently to compare in this way. The greatest change in the average T_2, maximum eigenvalue and mean eigenvalue profiles can be seen to occur in the superficial and transitional regions, i.e., the ∼40−50% of the cartilage closest

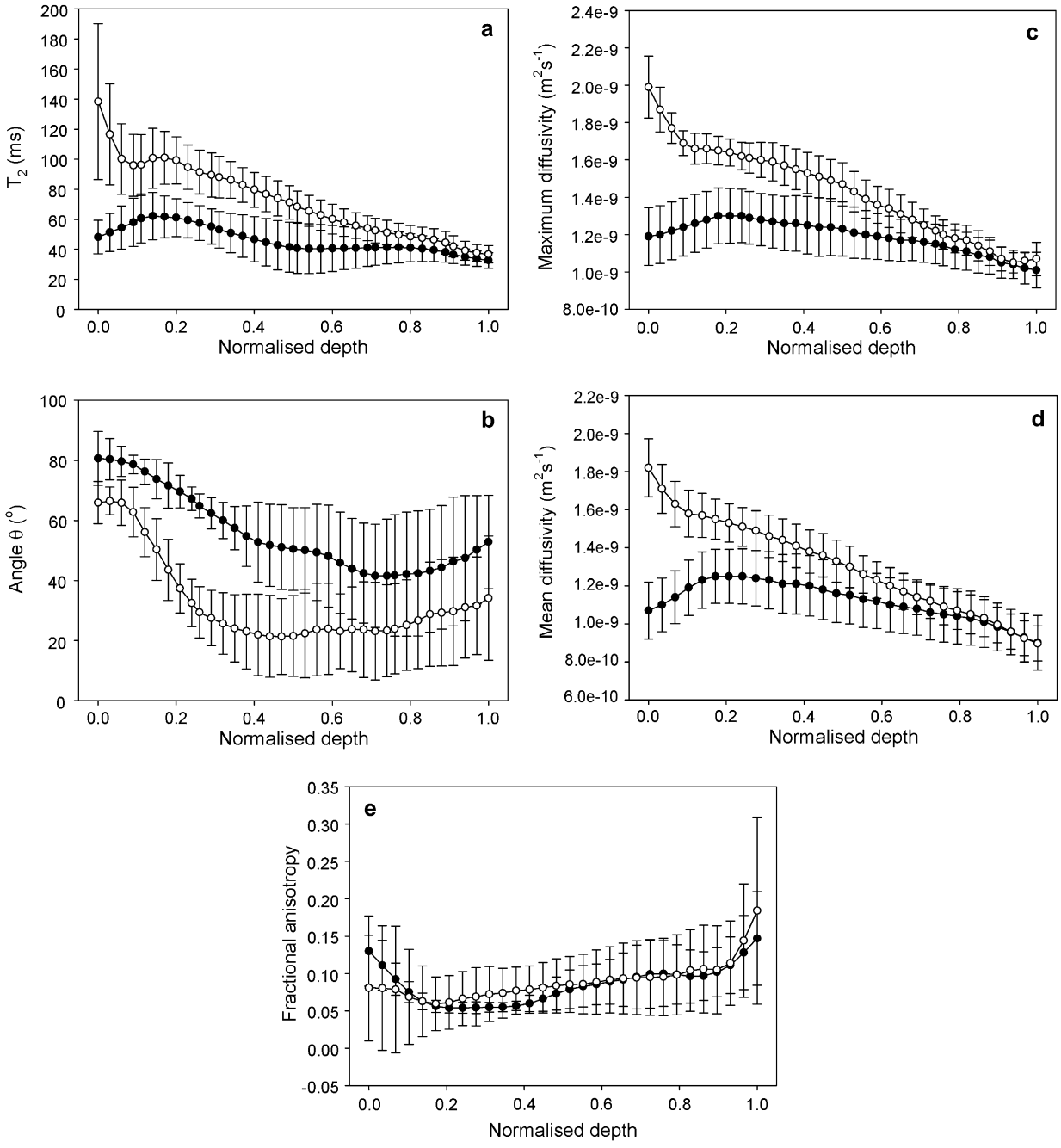

Fig. 3. Average profiles of (a) T_2, (b) principal eigenvector, (c) maximum eigenvalue, (d) mean eigenvalue and (e) fractional anisotropy, for the seven samples in this study that were compressed by 25% ± 5%. (○, before compression; ●, after compression) All samples were oriented with the normal to the articular surface at 55° ± 5° to the static magnetic field.

to the articular surface. For the profiles of principal eigenvector orientation θ, the greatest change occurs not at the surface but in the transitional zone between ~10% and 40% of the cartilage thickness from the surface.

From the data for each of the ten samples used in this study, values for the change in both T_2 and in the principal eigenvector orientation θ on compression were averaged across the full depth of the sample. These results (Fig. 4) were plotted against the percentage by which each sample was compressed. In some cases samples were compressed consecutively to two different strains, each contributing to the data points in the plots of Fig. 4. Both sets of data show a correlation with the degree of compression. The T_2 data fit a linear regression with an R^2 value of 0.60, while the principal eigenvector and principal eigenvalue data fit linear regressions with R^2 values of 0.81 and 0.78, respectively. The corresponding R^2 value for a linear fit to the corresponding plot for mean diffusivity was 0.74, whereas the average fractional anisotropy showed no correlation with degree of compression ($R^2 = 0.0025$).

Discussion

In this paper we report for the first time, changes in the water self-diffusion tensor arising from mechanical deformation of articular cartilage and compare the results with corresponding changes in the spin–spin relaxation time T_2, an MRI parameter that has previously been used to infer changes in collagen fibre orientation following compression[16].

In two previous studies (Meder *et al.*[4], Filidoro *et al.*[5]), it has been shown that the orientation of the principal component of the water diffusion tensor in cartilage (the principal eigenvector) can be correlated with the direction of the collagen fibre bundles. The results presented in this paper report similar principal eigenvector orientations in uncompressed cartilage to those in the previous studies. The principal eigenvector quiver plots (Fig. 2) and profiles [Fig. 3(b)] reflect the expected direction of the collagen fibre bundles in the uncompressed cartilage—approximately parallel to the articular surface near the surface, and approximately perpendicular to the bone in the lower half of the cartilage, with a transitional region between these peripheral and deep zones. The fact that the measured orientations of the principal eigenvectors in the peripheral zone (typically $\theta \sim 70^\circ$ with respect to the normal to the articular surface) and in the deep zone (typically $\theta \sim 20^\circ$) differ from the 'ideal' values of 90° and 0°, respectively, reflect an expected degree of disorder in the orientation of the collagen fibre bundles as well as noise in the diffusion tensor data[4].

The T_2 profiles presented in this report [Figs. 1(a) and 3(a)] can be compared with those from previous work by Alhadlaq and Xia[16]. Despite the difference in sample origin, (Alhadlaq and Xia used canine shoulder cartilage while this study used bovine knee cartilage), the shapes of both the uncompressed and compressed T_2 profiles reported here [Fig. 1(a)] are remarkably similar to those in the

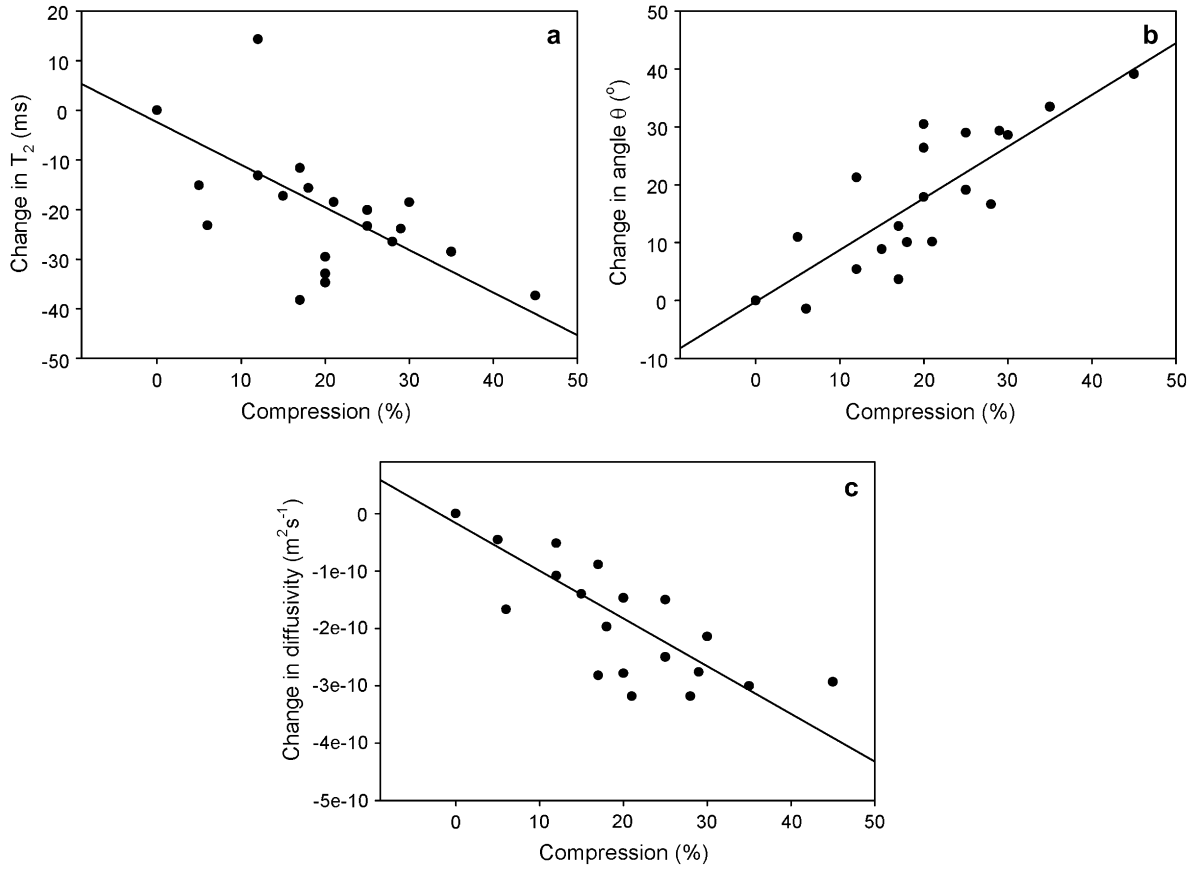

Fig. 4. Scatter plots with linear regression fits showing the observed changes in (a) T_2, (b) principal diffusion eigenvector orientation θ and (c) maximum diffusivity due to compression, plotted against the percentage compression of the sample. R^2 values for each of these regressions were (a) $R^2 = 0.60$ for T_2; (b) $R^2 = 0.81$ for the principal eigenvector orientation and (c) $R^2 = 0.78$ for the maximum diffusion eigenvalue.

previous study. Alhadlaq and Xia[16] used these T_2 profiles combined with previous results from PLM to present a model for the distortion of the collagen fibres on compression. However, the fact that T_2 is a complex function of both tissue water content and sample orientation in articular cartilage[7,9,10] complicates its interpretation as an indicator of collagen fibre orientation. In contrast, we believe that the water diffusion eigenvectors measured in this study have the potential to provide a more direct and less ambiguous indicator of changes in fibre orientation on compression.

Under compression, the average water diffusion eigenvalue orientation angle θ increases across the full depth of the sample [Fig. 3(b)], giving rise to a high correlation ($R^2 = 0.81$) between the degree of compression and the change in θ when averaged across the whole sample [see Fig. 4(b)]. In particular, the proportion of the cartilage depth over which the principal eigenvector is approximately parallel to the articular surface increases. This can also be seen in the quiver plots (Fig. 2). The change in θ is most noticeable in the transitional zone of the cartilage, where there is a change in average direction of the principal eigenvector by up to 40° [see Fig. 3(b)]. This suggests that compression leads to the greatest rearrangement of the collagen fibre bundles in the transitional zone, and that the rearrangement is such that the fibre bundles lie flatter with respect to the articular surface, leading to an increase in thickness of the superficial region. However, there is also an evidence of some increase in average orientation angle θ in both the peripheral and deep zones. In the peripheral zone this reflects a further flattening and consolidation of the fibres in this zone. In the deep zone, the increase in average orientation angle θ is consistent with some bending or crimping of the fibres in this zone. This interpretation is in broad agreement with previous studies that employed more invasive techniques[2,3,11,12].

The maximum eigenvalue and mean eigenvalue profiles [Fig. 3(c) and (d)] are very similar to those for T_2 [Fig. 3(a)] and show the greatest changes due to compression in the superficial and transitional regions. This can be correlated with the greatest water loss in these zones. Previous studies[20] have shown that the superficial and transitional regions are also the areas of highest water content in bovine cartilage. It is therefore to be expected that these zones will be compressed more (in relative terms) than the radial zone. However, despite this, the superficial region (in which the fibre orientation was predominantly parallel to the articular surface) appeared to increase in thickness (in both absolute and relative terms) on compression, indicating a fibre reorientation in this region of cartilage and consequent redefinition of the zone boundaries. This is in agreement with the results of Alhadlaq and Xia[16] based on T_2 data. The change in fractional anisotropy due to compression showed no correlation with the degree of compression, with R^2 values of 0.0025.

In order to investigate further the correlation between T_2 and DTI data, we have plotted the measured changes in maximum diffusivity [Fig. 5(a)] and in eigenvector orientation angle θ [Fig. 5(b)] as a function of the corresponding change in T_2. The results show good correlations between these parameters, with R^2 values of 0.76 and 0.59, respectively. While the DTI data provide a more direct measure of collagen fibre orientation, the fact that T_2 is easier to measure and less susceptible to motion artefacts may give it significant advantages as a parameter for use in clinical diagnosis, particularly if the relationship between collagen fibre architecture and T_2 anisotropy in cartilage can be modelled more precisely.

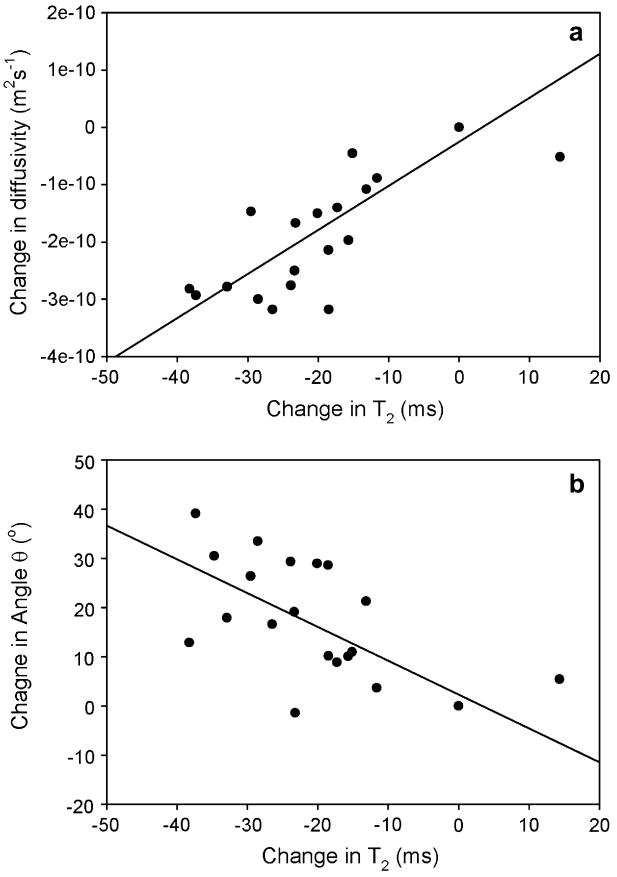

Fig. 5. Scatter plots with linear regression fits showing the observed changes in (a) principal diffusion eigenvalue and (b) principal diffusion eigenvector orientation angle θ due to compression, plotted against the corresponding change in T_2. R^2 values for each of these regressions were (a) $R^2 = 0.76$ and (b) $R^2 = 0.59$, respectively.

In summary, these results confirm that DTI can be used to probe the structural network of collagen fibres in cartilage, and provide new evidence of its ability to non-invasively detect changes induced by compression. Specifically the results show that articular cartilage responds to compression with a change in orientation of the collagen fibre bundles, most notably in the transitional zone, to become more aligned with the cartilage surface, together with a reduction in the water content, especially in the superficial and transitional zones. These results show that DTI is a powerful tool which can be used to gain a better understanding of the functional adaptations of articular cartilage. The technique may prove valuable in refining parameters employed in microstructural modelling of collagen network mechanics to better predict cartilage structure–function relationships[21]. It also has the potential to form the basis of a new non-invasive clinical tool for monitoring degenerative changes resulting from diseases such as osteoarthritis, leading to improved approaches to diagnosis and treatment of damaged tissue.

Acknowledgements

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